

Decontamination of *Bacillus* Spores Associated with Drinking Water Systems

*Biofilms are communities of microorganisms attached to surfaces that predominate in water/surface interfaces common to nearly all ecosystems. Biofilms offer a potential reservoir for dangerous organisms and also serve to protect these organisms from disinfectants. We have developed a PVC and copper pipe system to grow naturally occurring biofilms using well-characterized synthetic tap water containing humic acids. New and previously used pipes were studied for biofilm accumulation and spore retention. Once the biofilms were established, the pipes were subjected to *Bacillus thuringiensis* (BT) spores, a common simulant for *Bacillus anthracis*. Our initial studies focused on the use of bleach to inactivate the spores attached to the pipe surfaces and in the water.*

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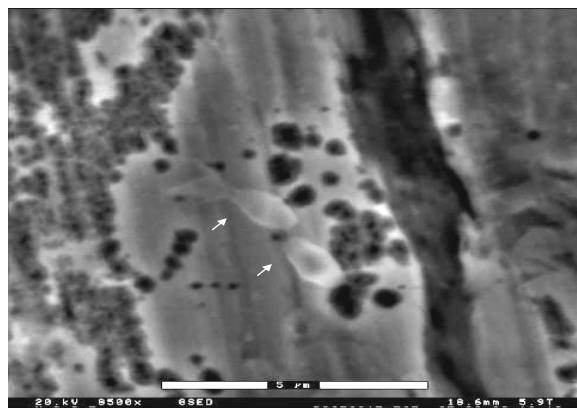
The NIST research team demonstrated that the rate of inactivation for *Bacillus thuringiensis* (BT) spores in the absence of the biofilm is higher than the rate for BT spores associated with biofilm when subjected to various concentrations of bleach. Free chlorine was not exhausted by the biofilm and viable spores were isolated from the pipe biofilms after a one-hour bleach treatment with free chlorine at a concentration of 12 mg/L. Biofilms harboring spores on PVC pipes were consistently inactivated with a higher efficiency than the same spores on copper pipes. The data showed that a much higher concentration of chlorine (12 mg/L to 120 mg/L compared to a normal residual of 1 to 3 mg/L) was necessary to remove the spores from the biofilms grown on commonly used pipe materials. Spores associated with biofilms grown on copper pipe surfaces were more resistant to free chlorine than spores on PVC pipes. The age and condition of these pipes also had an impact on biofilm concentration, spore retention, and disinfectant efficacy.

We are continuing this research to include ricin, *Escherichia coli*, and the bacteria that is the cause of tularemia, and also to study the effects of other methods of decontamination. We also plan to determine the fate of these

threats and the efficiency of decontamination in large-scale water systems. These results are part of an ongoing project to study the safety of building water systems in collaboration with researchers in the Building and Fire Research Laboratory.

The project is supported by the EPA Homeland Security Research Center. This research is part of a larger project that aims to collect information and develop guidelines that will aid in the decontamination of water systems in buildings in the event of contamination and to ensure the safety of our drinking water.

The objective of this work is to elucidate the fate of biological threats in drinking water systems and evaluate the efficacy of commonly used disinfectants for decontamination.



Bacillus thuringiensis spores associated with biofilm polysaccharide on a copper pipe surface. The Environmental Scanning Electron Microscopy (ESEM) images were collected at nearly 100% relative humidity. The arrows indicate spores. The bar length = 5 μ m.